

## Validation of a Noninvasive Assessment of Pulmonary Gas Exchange During Exercise in Hypoxia

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### **Validation of a Non-invasive Assessment of Pulmonary Gas Exchange During Exercise in Hypoxia**

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**Abbreviations**

- A-aDO<sub>2</sub>: Alveolar-to-arterial oxygen difference
- ABG: Arterial blood gas
- AGM100: MediPines Gas Exchange Monitor
- F<sub>I</sub>O<sub>2</sub>: Fraction of inspired oxygen
- gPaO<sub>2</sub>: Calculated partial pressure of arterial oxygen via non-invasive methods
- P<sub>50</sub>: Partial pressure of oxygen associated with 50% oxygen saturation
- P<sub>A</sub>O<sub>2</sub>: Alveolar partial pressure of oxygen
- P<sub>Atm</sub>: Atmospheric pressure
- PaO<sub>2</sub>: Partial pressure of arterial oxygen
- PCO<sub>2</sub>: Partial pressure of carbon dioxide
- P<sub>ET</sub>O<sub>2</sub>: Partial pressure of end-tidal oxygen
- PH<sub>2</sub>O: Partial pressure of water
- PO<sub>2</sub>: Partial pressure of oxygen
- RER: Respiratory exchange ratio
- SaO<sub>2</sub>: Oxygen saturation of arterial haemoglobin
- SpO<sub>2</sub>: Oxygen saturation of peripheral capillary

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**Abstract:**

**Background:** Pulmonary gas exchange efficiency, determined by the alveolar-to-arterial PO<sub>2</sub> difference (A-aDO<sub>2</sub>), progressively worsens during exercise at sea-level; this response is further elevated during exercise in hypoxia. Traditionally, pulmonary gas exchange efficiency is assessed through measurements of ventilation and end-tidal gases paired with direct arterial blood gas (ABG) sampling. Since these measures have a number of caveats, particularly invasive blood sampling, the development of new approaches for the non-invasive assessment of pulmonary gas exchange is needed.

Research Question: Is a non-invasive method of assessing pulmonary gas exchange valid during rest and exercise in acute hypoxia?

**Study Design and Methods:** Twenty-five healthy participants (10 female) completed a staged maximal exercise test on a cycle ergometer in a hypoxic chamber (F<sub>I</sub>O<sub>2</sub>=0.11). Simultaneous ABGs via a radial arterial catheter and non-invasive gas-exchange measurements (AGM100) were obtained in two-minute intervals. Non-invasive gas exchange, termed the O<sub>2</sub> deficit, was calculated from the difference between the end-tidal and the calculated PaO<sub>2</sub> (via pulse oximetry and corrected for the Bohr effect by using the end-tidal PCO<sub>2</sub>). Non-invasive O<sub>2</sub> deficit was compared to the traditional alveolar to arterial oxygen difference (A-aDO<sub>2</sub>) using the traditional Riley analysis.

**Results:** Under conditions of rest at room air, hypoxic rest and hypoxic exercise, strong correlations between the calculated gPaO<sub>2</sub> and directly measured PaO<sub>2</sub> (R<sup>2</sup>=0.97; p<0.001; mean bias =1.70 mmHg) were observed. At hypoxic rest and exercise, strong relationships between the estimated and directly measured PaO<sub>2</sub> (R<sup>2</sup>=0.68; p<0.001; mean bias =1.01mmHg) and O<sub>2</sub> deficit with the traditional A-aDO<sub>2</sub> (R<sup>2</sup>=0.70; p<0.001; mean bias =5.24mmHg) remained.

**Interpretations:** Our findings support the use of a non-invasive measure of gas exchange during acute hypoxic exercise in healthy humans. Further studies are required to determine if this approach can be used clinically as a tool during normoxic exercise in patients with pre-existing impairments in gas exchange efficiency.

## 61 INTRODUCTION:

62 It is well established that pulmonary gas exchange efficiency, determined by the alveolar-to-  
63 arterial  $PO_2$  difference ( $A-aDO_2$ ) progressively worsens (i.e., the  $A-aDO_2$  increases) in a  
64 workload-dependent manner during exercise in normoxia due to both pulmonary factors (a  
65 combination of alveolar ventilation-to-perfusion inequalities, diffusion limitation, and  
66 intrapulmonary right-to-left shunt) and non-pulmonary factors (i.e., extrapulmonary right-to-left  
67 shunt) [reviewed in: <sup>1</sup>]. This impairment in pulmonary gas exchange efficiency during exercise is  
68 further exacerbated in acute hypoxia, such that for any given oxygen consumption, the  $A-aDO_2$  is  
69 greater when compared to exercise in normoxic conditions resulting in further hypoxemia <sup>2</sup>.

71 The current gold-standard for assessing changes in gas exchange during exercise and/or in  
72 pathological conditions is via invasive sampling of arterial blood gases (ABG). However, there  
73 are several technical and logistical limitations that make the acquisition of these samples  
74 difficult; time to complete sampling & processing procedure; repeated blood sampling that may  
75 increase risk of infection and/or anemia; variability in the measure; requirement of a technically  
76 skilled operator; confinement to a laboratory or hospital; damage to the endothelium during  
77 catheter insertion <sup>3</sup>; possible anxiety during cannulation; and limitations of the traditional ideal  
78  $A-aDO_2$  calculations relating to the traditional Riley method of measuring alveolar  $O_2$  [reviewed  
79 in: <sup>4</sup>]. For these reasons, there has been recent progress in a noninvasive method utilizing the  
80 MediPines Gas Exchange Monitor (AGM100)<sup>1</sup> for assessing impaired pulmonary gas exchange  
81 <sup>5-8</sup>. This method employs measuring end-tidal  $PO_2$  and  $PCO_2$  from the expired gas during steady-  
82 state breathing and then deriving the arterial  $PO_2$  via pulse oximetry. Allowing for the Bohr  
83 effect by use of the end-tidal  $PCO_2$ , the difference between the end-tidal, which is assumed to  
84 equal alveolar  $PO_2$ , and the calculated  $PO_2$  is defined as the oxygen deficit ( $O_2$  deficit).  
85 Collectively, these studies have confirmed that this non-invasive procedure is a highly sensitive  
86 indicator of impaired pulmonary gas exchange in lung disease <sup>5</sup>. Additionally, in resting  
87 conditions in elderly healthy volunteers and in patients with lung disease, the estimated oxygen  
88 deficit correlates well with the directly measured  $A-aDO_2$  <sup>5,7,8</sup>. Despite the promising  
89 developments in this non-invasive procedure, it has not been established if the AGM100 can

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<sup>1</sup> The MediPines Gas Exchange Monitor was cleared by the US FDA

accurately detect changes in pulmonary gas exchange efficiency during exercise, particularly under hypoxic conditions where substantial increases in the A-aDO<sub>2</sub> occur resulting in further arterial hypoxemia.

The aim of this study was to compare the predicted arterial PO<sub>2</sub> and oxygen deficit from the non-invasive method with directly measured arterial blood gases at rest and during graded exercise in acute hypoxia. We examined the related hypotheses that the non-invasive measures would be strongly related to the direct arterial blood gas assessments of pulmonary gas exchange efficiency.

**METHODS**

***Ethical approval***

All participants gave written informed consent prior to participating. This study was approved by the University of British Columbia Clinical Research Ethics Board (H17-02687-A008) and conformed to the standards set by the *Declaration of Helsinki* (except registry in a database) and the Canadian Government Tri-council Policy Statement (TCPS2) for integrity in research.

***Participants:***

Fifteen male (28.1±6.2 years; body mass 77.2±9.4 kg; height 178.6±5.2 cm; BMI 24.2±2.5 kg/m<sup>2</sup>) and ten female (24.9±3.6 years; body mass 63.1±3.9 kg; height 170.0±5.0 cm; BMI 21.9±1.7 kg/m<sup>2</sup>) participants were recruited from the University of British Columbia Okanagan (344 m elevation). All participants were healthy and free from respiratory or cardiovascular disease, did not smoke, and self-reported engaging in 3-5hrs of moderate/vigorous physical activity per week. Participants were instructed to refrain from caffeine and alcohol for 12 hrs and strenuous exercise for 24 hrs before testing.

***Experimental overview***

While resting in the supine position a radial artery catheter (20-gauge; Arrow, Markham, ON, Canada) was inserted into the left radial artery under local anesthesia (Lidocaine, 1.0%) and ultrasound guidance. The arterial catheter was attached to an inline waste-less sampling system (Edwards Lifesciences, TruWave VAMP, CA, USA). Following cannulation, participants entered a normobaric hypoxia chamber ( $F_{I}O_2 = 0.11$ ) for 30-minutes. Following a 30-minute period of seated rest (in hypoxia), participants completed an incremental cycling test to exhaustion on an up-right cycle ergometer (Excalibur Sport, Lode, London, UK). To match relative exercise time between sexes, male participants began cycling at 20 watts, increasing by 30 watts every 2 minutes, while females began cycling at 15 watts, increasing by 20 watts every 2 minutes. All participants were instructed to cycle at a comfortable cadence (between 50 and 80 RPM) on an individual basis and to exercise until maximal volitional exhaustion. Subjects were asked to breathe through a small mouthpiece connected to a galvanic  $O_2$  and infrared  $CO_2$  analyzer, and were fitted with a pulse oximeter (Unimed U405S-06 Reusable Soft Finger Sensor, Unimed, Shenzhen, China) for AGM100 (MediPines Corporation, Yorba Linda, USA) measurements both at rest and throughout the exercise test. Simultaneous AGM100 measurements and arterial ABG samples were obtained during normoxic rest prior to entering the hypoxia chamber, during hypoxic rest, and in the last 30 seconds of each exercise stage. From each blood gas sample, any air bubbles were immediately evacuated from the syringe, and blood analysis was performed within 10-minutes of sampling with a commercially available gas analyzer (ABL90 FLEX, Radiometer, Copenhagen, Denmark). The blood gas analyzer was calibrated at a minimum of every 8 hours using manufacturer's standard internal quality checks. Reported variables that were calibrated and analyzed included  $PaO_2$ ,  $PaCO_2$ , pH and oxygen saturation of arterial oxyhaemoglobin ( $SaO_2$ ). For the present study, the within day coefficient of variation of measurement for  $PaO_2$  was 1.5% and 2.1% for  $PaCO_2$ , respectively. The between-day coefficient of variation of the main variables (end-tidal gases,  $SpO_2$  and  $O_2$  deficit) of the AGM100 following exposure to normobaric hypoxia ( $F_{I}O_2 = 0.12$ ) was <5%.

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#### 144 *Non-invasive gas exchange monitoring*

145 Non-invasive gas-based assessment of arterial  $PaO_2$  ( $gPaO_2$ ) was obtained by converting  $SpO_2$   
 146 obtained from the finger pulse-oximeter using the Hill equation as shown below:

$$147 \quad PaO_2^n = (P_{50}^n) \times [SpO_2 / (1 - SpO_2)] \quad \text{Eq 3.}$$



Whereby  $P_{50}$  represents the  $PO_2$  associated with 50% oxygen saturation (27mmHg), and  $n$  represents a constant of 2.7. The  $P_{50}$  was adjusted by the measured end-tidal  $PCO_2$ <sup>9,10</sup>. Simultaneous assessment of end-tidal gases allows for  $PCO_2$  associated changes in the oxygen affinity of hemoglobin to be accounted for using the Kelman subroutine. However, it is not possible to allow for changes in pH caused by alterations in base excess, as discussed previously<sup>5-8</sup>. Although the AGM100 software, by default, averages breath data over the previous eight breaths to get steady normalized sampling, for this study, gas exchange data were averaged over 20-seconds at the end of each stage to limit the influence of ventilatory artifacts on influencing the measure. It has been previously stated that it is important to capture “steady state” values during each non-invasive session<sup>4</sup>. During steady-state respiration, a dynamic equilibrium exists between oxygen and carbon dioxide and averaged values are highly reproducible as they are derived from the lung at functional residual capacity.

Alveolar  $PO_2$  ( $P_{AO_2}$ ) was calculated via the alveolar gas equation<sup>11</sup> and coupled with direct arterial  $PaO_2$  measurements for the standard assessment of A-a $DO_2$  gradient to compare against the non-invasive  $O_2$  deficit measure:

$$P_{AO_2} = (P_{Atm} - PH_2O) \times F_{IO_2} - P_{ACO_2} \times [F_{IO_2} + ((1-F_{IO_2})/RER)] \quad \text{Eq 4.}$$

Where  $P_{Atm}$  and  $PH_2O$  are the barometric pressure and water vapor pressure, respectively;  $P_{AO_2}$  and  $P_{ACO_2}$  are the alveolar partial pressures of oxygen and carbon dioxide, respectively;  $F_{IO_2}$  is the fraction of inspired oxygen; RER is the respiratory exchange ratio. RER was estimated using linear regression whereby RER was assumed to equal 0.80 at rest and 1.10 during the final stage of exercise.

The traditional A-a $DO_2$  was calculated from  $P_{AO_2} - PaO_2$  obtained from equation 4 above and from the ABG.  $O_2$  deficit assessed by the AGM100 was determined to be the difference from  $P_{AO_2} - gPaO_2$  whereby  $P_{AO_2}$  is assumed to equal end-tidal  $O_2$  and  $gPaO_2$  is calculated as described earlier. A third method of assessing pulmonary gas exchange efficiency was also employed to account for variability in the traditional Riley method of assessing  $P_{AO_2}$ . Termed the “modified method”, A-a $DO_2$  was calculated as  $P_{AO_2} - PaO_2$  whereby  $P_{AO_2}$  is assumed to equal end-tidal  $PO_2$  as is the case with the non-invasive method, while  $PaO_2$  is obtained from the direct arterial sample.

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### 178 ***Data Statistical Analyses***

179 Statistical analyses were performed using Prism Graphpad 8, IBM SPSS Statistics Version 24, and  
180 R statistical software. Linear regression and Bland–Altman analysis was performed <sup>11</sup> using Prism  
181 Graphpad to evaluate the agreement between the estimated arterial PO<sub>2</sub> and oxygen deficit from  
182 the non-invasive method with directly measured ABGs at rest and during graduated exercise in  
183 acute hypoxia. Further, repeated measures correlation analysis <sup>13</sup> were performed using R (rmcorr  
184 package Version 0.3.0; <https://cran.r-project.org/web/packages/rmcorr/>) to account for inter-  
185 individual variability. Mean bias and precision ( $\pm 1$  SD) are reported using the Bland-Altman plots  
186 whereby the difference between the non-invasive measure and “gold standard” (Y-axis) are plotted  
187 against the “gold standard” (X-axis). Potential sex differences between males and females during  
188 exercise were evaluated using a linear mixed model in SPSS with sex and exercise stage assigned  
189 as fixed effects and participants as random effects. Reported p-values are two tailed with  
190 significance set at  $P \leq 0.05$  for all statistical tests.

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### 193 **RESULTS**

194 Twenty-five volunteers were included in this study, yielding N=206 data points for comparison  
195 between AGM100 and ABG. Oxygen deficit during rest and exercise in males and females are  
196 presented in Figure 1A & 1B. Linear regression data comparing non-invasive and directly  
197 measured PO<sub>2</sub> in males and females during rest and all exercise time points in hypoxia are  
198 presented in Figure 2A. Under combined conditions of normoxic rest, hypoxic rest and hypoxic  
199 exercise, the results revealed strong correlations between the calculated gPaO<sub>2</sub> and directly  
200 measured PaO<sub>2</sub> ( $R^2=0.97$ ;  $p<0.001$ ; mean bias =1.70 mmHg). During rest in normoxic  
201 conditions, a moderate correlation was observed across all participants ( $R^2=0.29$ ;  $p=0.013$ ) with  
202 low mean bias and relatively large variability (mean bias= -0.95 $\pm$ 9.33 mmHg). At hypoxic rest  
203 and exercise, the strong correlations between gPaO<sub>2</sub> and ABG PaO<sub>2</sub> ( $R^2=0.68$ ;  $p<0.001$ )  
204 remained with no statistical differences between sexes ( $P=0.99$ ) (males:  $R^2=0.69$ , females  
205  $R^2=0.62$ ; both  $p<0.001$ ). This relationship remained present when analyzed using repeated

measures correlation ( $R^2=0.59$ ;  $p<0.001$ ). When compared against rest conditions in hypoxia, the correlation was also very strong ( $R^2=0.85$ ;  $p<0.001$ ). However, if resting data is excluded, the strength of correlation was reduced (males:  $R^2=0.53$ , females:  $R^2=0.46$ ; both  $p<0.001$ ). Although the  $O_2$  deficit was not different at maximal exercise, females exhibited greater arterial hypoxemia (both ABG  $PaO_2$  and  $gPaO_2$  were lower) than males across exercise trials ( $p<0.001$ ). Bland-Altman analysis are presented in Figure 2B in the corresponding right-hand panel. There was low mean bias between  $gPaO_2$  and  $PaO_2$  ( $0.96\pm2.75$  mmHg) during rest and all exercise stages in hypoxia with minimal differences between sexes (males= $0.80$  mmHg, females= $1.17$  mmHg).

The A-a $DO_2$  and oxygen deficit data are compared in Figure 2C and 2D. Although the conventional A-a $DO_2$  gradient measure and  $O_2$  deficit show both a strong correlation ( $R^2=0.71$ ;  $P<0.001$ ) and repeated measures correlation ( $R^2=0.74$ ),  $O_2$  deficit was reflected in a positive mean bias ( $5.24\pm4.96$  mmHg). Part of the positive mean bias was evident in the lower A-a $DO_2$  values at each exercise stage, including negative values during rest and light exercise. Finally, as displayed in Figure 3, the mixed method utilizing  $P_{ET}O_2$  rather than the Riley estimated alveolar  $O_2$  (via Eq 4.) to calculate A-a $DO_2$  presents similar values compared to  $O_2$  deficit ( $R^2=0.87$ ; mean bias= $1.72\pm2.69$  mmHg).

**DISCUSSION**

The purpose of this study was to test the validity and reliability of a non-invasive method for assessing pulmonary gas exchange at rest and during exercise in acute hypoxia. The main findings of this investigation were the following: Despite the modest results in resting room air conditions, strong relationships and low mean bias were observed during hypoxic rest and exercise between the calculated  $gPaO_2$  and directly measured  $PaO_2$  ( $R^2=0.68$ ;  $p<0.001$ ; mean bias= $1.01$  mmHg) and  $O_2$  deficit with A-a $DO_2$  ( $R^2=0.71$ ;  $p<0.001$ ; mean bias= $5.24$  mmHg). Moreover, a small yet significant difference in exercise-induced hypoxemia was also revealed whereby females presented with greater degree of arterial hypoxemia across rest and exercise that was detected in both directly measured and calculated  $PaO_2$ . The following discussion

considers the evidence, experimental limitations and the relevance underlying the findings of this study.

### ***Validation during exercise***

During exercise in acute hypoxia, albeit with a positive mean bias of ~5 mmHg, the non-invasive AGM100 was strongly correlated with that of the classic A-aDO<sub>2</sub> measure. Exercise intensity dependent increases in O<sub>2</sub> deficit in both male and female participants occurred following a similar pattern of pulmonary gas exchange impairment compared to previous hypoxic exercise studies using invasive techniques [e.g., <sup>2,14,15</sup>]. Moreover, during rest and exercise in acute hypoxia the AGM100 estimated gPaO<sub>2</sub> was strongly correlated with PaO<sub>2</sub> and exhibited low mean bias (<3 mmHg). This finding is consistent with previous reports at rest in healthy participants in acute hypoxia <sup>6</sup> and in patients with chronic obstructive pulmonary disease <sup>5</sup>. Improvements in pulse oximeter design and related signal detection algorithms may explain our improved results compared to previous reports during exercise <sup>16</sup>. A further important observation was that the AGM100 detected a significant difference in gPaO<sub>2</sub> during exercise whereby female participants exhibited a greater degree of hypoxaemia during exercise. This is not a novel finding in itself and is likely due to differences in lung volume and airway diameter [reviewed in; <sup>17</sup>], work of breathing <sup>18</sup>, and mechanical ventilatory constraint between sexes <sup>19,20</sup> however, this finding and the high reproducibility of measures (i.e., between-day coefficient of variation of O<sub>2</sub> deficit in hypoxia of <5%), highlights the sensitivity of the non-invasive AGM100 to detect subtle differences in pulmonary gas exchange between even healthy volunteers.

Although the classic A-aDO<sub>2</sub> method is considered the current “gold standard” and is used extensively in research for assessing pulmonary gas exchange both at rest and during exercise, it is not without its caveats. Indeed, it is not uncommon to observe negative A-aDO<sub>2</sub> values at rest or during light exercise [reviewed in: <sup>1</sup>], a physiologically improbable finding. This was also the case in our study whereby utilizing the classic A-aDO<sub>2</sub> equation exhibited a mean negative gradient at rest and low intensity exercise in acute hypoxia in 59 out of 203 sample points. Linear regression analysis between the classic A-aDO<sub>2</sub> equation and the non-invasive O<sub>2</sub> deficit measure

revealed a strong correlation between the two measures (see Fig 2C and 2D); however, O<sub>2</sub> deficit exhibited a relatively higher mean bias, eliminating the physiologically improbable negative gradient, though presenting with greater levels of impaired gas exchange during strenuous exercise. Utilization of a “modified method” A-aDO<sub>2</sub> equation whereby P<sub>ET</sub>O<sub>2</sub> was assumed to equal alveolar O<sub>2</sub> - as is the case with the AGM100 - eliminated the occurrence of negative pulmonary gradient values. Thus, the possible sources of error in the traditional method are likely resultant of issues with the calculation of P<sub>A</sub>O<sub>2</sub> utilizing Riley analysis and can be divided into two possible areas of concern that should be considered. 1) variability in the values for alveolar PCO<sub>2</sub> acquired from the arterial sample and 2) the ventilatory parameters which are acquired non-invasively and are not physiologically produced or rely on certain assumptions including F<sub>I</sub>O<sub>2</sub> and respiratory quotient. Utilizing single timepoint arterial sampling to assess pulmonary gas exchange during an exercise stage is likely highly susceptible to ventilatory artifacts and sampling time discrepancies between the arterial and ventilatory variables required for the classic A-aDO<sub>2</sub> equation whereby brief episodes of altered ventilation may result in transient fluctuations in PaCO<sub>2</sub><sup>5</sup>. Indeed, lack of steady state conditions through anxiety induced hyperventilation at rest while seated on a cycle ergometer have previously been suggested to cause negative A-aDO<sub>2</sub> values<sup>1</sup>. This observation indicates that this lack of steady-state may be exacerbated under acute hypoxic conditions whereby marked hyperventilation and related arterial hypocapnia are elicited<sup>20</sup>. As the non-invasive AGM100 measurement utilized a 20s average of each exercise stage for the assessment of gPaO<sub>2</sub> and O<sub>2</sub> deficit, it is likely less influenced by acute ventilatory artifacts which may occur during exercise or in hypoxia whereby a “steady-state” is difficult to obtain unless averaged over a period of time. Further, as discussed previously<sup>5</sup>, the traditional A-aDO<sub>2</sub> equation is heavily favoured towards regions of the lung with abnormally low ventilation-perfused ratios whereas end-tidal PO<sub>2</sub> values represent a more uniform distribution of ventilation-perfused areas of the lung.

**Methodological Considerations**

The AGM100 has emerged to be effective for the non-invasive quantification of abnormal pulmonary gas exchange<sup>5-8</sup>; however, this approach cannot differentiate between the contributing mechanisms of impaired gas exchange (e.g., diffusion limitation, hypoventilation,

ventilation/perfusion heterogeneity and shunting). Although different approaches (e.g., multiple inert gas elimination techniques, etc.) may provide insight into these factors, it is important to note that no assessment is perfect, and the AGM100 assessment provides a simple, non-invasive method for assessing pulmonary gas exchange, via  $O_2$  deficit, in a time conscious and easily repeatable manner <sup>4</sup>. Moreover, the method in which the  $O_2$  deficit is calculated overcomes several limitations of the classic Riley analysis of the A-a $DO_2$ , including: 1) lung units with high V/Q are included in the analysis rather than only units with abnormally low V/Q <sup>5</sup>; 2) it provides instantaneous calculations of gPa $O_2$ ; 3) the  $O_2$  deficit is completely non-invasive; and, on the basis of the current study, 4) the calculation of gPa $O_2$  and  $O_2$  deficit remain valid during exercise. Of note, some limitations of the AGM100 device have been elegantly reviewed <sup>22</sup>. The relevant points in the context of our experimental design are as follows. First, the Pa $O_2$  calculations are based on the subroutines developed by Kelman to correct for the allosteric effect of changes in PCO<sub>2</sub> on the oxygen affinity of hemoglobin, known as the Bohr effect <sup>9,10</sup>. The Kelman's solutions assume that the change in PCO<sub>2</sub> shifts the arterial PO<sub>2</sub> along the normal buffer line. Second, given the shape of the oxyhemoglobin dissociation curve is flatter at the upper range of oxygen saturation, the variability of gPaO<sub>2</sub> widens at the SpO<sub>2</sub> >97% as indicated by the weaker relationship ( $R^2=0.3$ ) observed in our study. Third, the same considerations of the effects of temperature on gPa $O_2$  also remain unknown. Our validation data during exercise support the absence of a major influence of temperature on gPa $O_2$  and  $O_2$  deficit. Moreover, recent mathematical simulations suggest that the influence of both temperature and base excess on calculated gPa $O_2$  are modest (e.g., <3mmHg) <sup>4</sup>. Although we would not anticipate any compensatory alterations in acid-base balance in the current acute study, the changes in core (esophageal) temperature with acute exercise are also small (<0.5 °C).

## CONCLUSION

This study found that pulmonary gas exchange efficiency measured using a non-invasive gas exchange monitor provided a valid and reliable measure against directly measured arterial blood gasses during hypoxic exercise. Further, the non-invasive oxygen deficit was strongly correlated with A-a $DO_2$  values obtained from the classic A-a $DO_2$  equation without presenting with negative  $O_2$  gradient values. These results provide promising evidence to support the use of non-invasive



gas exchange assessments during hypoxic exercise which may be applicable to both laboratory and clinical patient assessments. Further studies are now required to determine if this approach can be used clinically as a tool during normoxic exercise in patients with pre-existing impairments in gas exchange efficiency.

**Author contributions**

C.A.H., D.B.M., L.W., S.J.O., and P.N.A. were involved in data collection. C.A.H. and P.N.A. were involved in data analyses and interpretation. C.A.H. and P.N.A. drafted the manuscript. All authors critically reviewed the manuscript. C.A.H. and P.N.A. conceived the study design. All authors approved the final version of this manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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**Figure Legends**

**Figure 1:** Oxygen deficit ( $O_2$  deficit) during rest and cycling exercise during stages 1-9 of an incremental maximal exercise test in normobaric hypoxia ( $F_{I}O_2=0.11$ ). Figure 1A; Data are presented as individual data points normalized to percent of maximal workload with lines of best fit. Male participants are represented by blue open circles with a solid line and female participants are represented by red open circles with a dashed line. Figure 1B; Data are presented as group means  $\pm$  SD for each absolute workload. Male participants (blue bars) began cycling at 20 watts, increasing by 30 watts every two minute while females (red bars) cycled at 15 watts, increasing by 20 watts every two minutes. The total number of exercise stages completed are as follows: six stages= 1 male, 1 female; seven stages= 5 males, 4 females; eight stages= 7 males, 4 females; nine stages= 2 males, 1 female.

**Figure 2:** Linear regression and Bland-Altman figures for gas exchange monitor assessed partial pressure of arterial oxygen (gPaO<sub>2</sub>) vs arterial blood gas assessed partial pressure of arterial oxygen (ABG PaO<sub>2</sub>) [A,B] and oxygen deficit vs the traditional alveolar to arterial difference of oxygen (A-aDO<sub>2</sub>) [C,D] presented as individual data points at hypoxic rest and at all stages of hypoxic exercise. Male data points are indicated by blue open circles and solid linear regression lines. Female data points are indicated by red open circles and dashed linear regression lines. R<sup>2</sup> and P-values presented in the figures represent both male and female data points combined. The single solid line and dotted lines in the Bland-Altman plots represent mean bias and  $\pm$  SD respectively.

**Figure 3.** Pulmonary gas exchange efficiency displayed as the 1) Traditional A-aDO<sub>2</sub> (black line); calculated using estimated alveolar O<sub>2</sub> from Riley analysis and directly measured partial pressure of arterial oxygen (PaO<sub>2</sub>) via arterial catheterization, 2) O<sub>2</sub> deficit (dark grey line); non-invasively assessed using estimated alveolar PO<sub>2</sub> from end-tidal PO<sub>2</sub> and calculated PaO<sub>2</sub>. 3) A mixed method A-aDO<sub>2</sub> (light grey line); assessed using estimated alveolar O<sub>2</sub> from end-tidal O<sub>2</sub> and directly measured PaO<sub>2</sub> via arterial catheterization. Data are presented as group means  $\pm$  SD during rest and cycling exercise during an incremental maximal exercise test in normobaric hypoxia (F<sub>I</sub>O<sub>2</sub>=0.11).



385   **REFERENCES**

386   1.     Stickland MK, Lindinger MI, Olfert IM, Heigenhauser GJF, Hopkins SR. Pulmonary Gas  
387         Exchange and Acid-Base Balance During Exercise. *Compr Physiol*. 2013;3:693-739.

388   2.     Wagner PD, Gale GE, Moon RE, et al. Pulmonary gas exchange in humans exercising at  
389         sea level and simulated altitude. *J Appl Physiol*. 1986;61:260-270.

390   3.     Dawson EA, Rathore S, Cable NT, Wright DJ, Morris JL, Green DJ. Impact of catheter  
391         insertion using the radial approach on vasodilatation in humans. *Clin Sci*  
392         2010;118(10):633–640.

393   4.     Prisk GK, West JB. Deriving the Arterial PO<sub>2</sub> and Oxygen Deficit from Expired Gas and  
394         Pulse Oximetry. *J Appl Physiol*. 2019;127(4):1067-1074

395   5.     West JB, Crouch DR, Fine JM, Makadia D, Wang DL, Prisk GK. A New, Noninvasive  
396         Method of Measuring Impaired Pulmonary Gas Exchange in Lung Disease: An Outpatient  
397         Study. *Chest*. 2018;154:363-369.

398   6.     West JB, Wang DL, Prisk GK. Measurements of pulmonary gas exchange efficiency  
399         using expired gas and oximetry: results in normal subjects. *Am J Physiol Lung Cell Mol*  
400         *Physiol*. 2018;314:686-689.

401   7.     West JB, Prisk GK. A new method for noninvasive measurement of pulmonary gas  
402         exchange using expired gas. *Respir Physiol Neurobiol*. 2018;247:112-115.

403   8.     West JB, Wang DL, Prisk GK, et al. Noninvasive measurement of pulmonary gas  
404         exchange: comparison with data from arterial blood gases. *Am J Physiol Cell Mol Physiol*.  
405         2018;316(1):L114-L118.

406   9.     Kelman Richard G. Computer Program for the Production of O<sub>2</sub>-CO<sub>2</sub> Diagrams. *Respir*

- 407        *Physiol.* 1968;4:260-269.
- 408    10.    Kelman RG. Calculation of Certain Indices of Cardio-pulmonary Function Using a Digital  
409        Computer. *Respir Physiol.* 1966;1:335-343.
- 410    11.    Riley RL, Cournand A. 'Ideal' Alveolar Air and the Analysis of Ventilation-Perfusion  
411        Relationships in the Lungs. *J Appl Physiol.* 1949;1(12):825-847.
- 412    12.    Bland JM, Altman DG. Statistical methods for assessing agreement between two methods  
413        of clinical measurement. *Lancet.* 1986;327:307-310.
- 414    13.    Bakdash JZ, Marusich LR. Repeated Measures Correlation. *Front Psychol.* 2017;8.
- 415    14.    Torre-bueno R, Wagner PD, Saltzman HA, Gale GE, Moon F G RE, Moon RE. Diffusion  
416        limitation in normal humans during exercise at sea level and simulated altitude. *J Appl*  
417        *Physiol.* 1985;58(3):989-995.
- 418    15.    Wagner PD, Sutton JR, Reeves JT, Cymerman A, Groves BM, Malconian MK. Operation  
419        Everest II: pulmonary gas exchange during a simulated ascent of Mt. Everest. *J Appl*  
420        *Physiol.* 1987;63(6):2348-2359.
- 421    16.    Yamaya Y, Bogaard HJ, Wagner PD, Niizeki K, Hopkins SR. Validity of pulse oximetry  
422        during maximal exercise in normoxia, hypoxia, and hyperoxia. *J Appl Physiol.*  
423        2002;92(1):162-168.
- 424    17.    Harms CA. Does gender affect pulmonary function and exercise capacity? *Respir Physiol*  
425        *Neurobiol.* 2006;151(2-3):124-131.
- 426    18.    Dominelli PB, Molgat-Seon Y, Bingham D, et al. Dysanapsis and the resistive work of  
427        breathing during exercise in healthy men and women. *J Appl Physiol.* 2015;119(10):1105-  
428        1113.

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429 19. Wanke T, Formanek D, Schenz G, Popp W, Gatol H, Zwick H. Mechanical load on the  
430 ventilatory muscles during an incremental cycle ergometer test. *Eur Respir J.* 1991;4:385-  
431 392.

432 20. Guenette JA, Witt JD, McKenzie DC, Road JD, Sheel AW. Respiratory mechanics during  
433 exercise in endurance-trained men and women. *J Physiol.* 2007;581(Pt 3):1309-1322.

434 21. Hoiland RL, Howe CA, Coombs GB, Ainslie PN. Ventilatory and cerebrovascular  
435 regulation and integration at high-altitude. *Clin Auton Res.* 2018;28(4):423-435.

436 22. Philipp X, Pickerodt A, Kuebler WM. Go West: translational physiology for noninvasive  
437 measurement of pulmonary gas exchange in patients with hypoxemic lung disease. *Am J*  
438 *Physiol Cell Mol Physiol.* 2019;316(5):L701-L702.





